EXPERIMENTS ON THE ORIGIN OF THE CLEAVAGE CENTROSOMES.

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The great diversity of opinion as to the origin of the cleavage centrosomes which appear during the fecundation of the egg is well known to all students of cytology. By different authors every possible view has been held with regard to the source of these centers, as may be seen from the following classification:

1. The cleavage centrosomes come from the sperm centrosome; Boveri ('87, '92, '95), Vejdovsky ('88), Fick ('93), Wilson and Mathews ('95), Hill ('95), Mead ('95), Reinke ('95), Kostanecki and Wierzejski ('96), Kostanecki and Siedlecki ('96), Sobotta ('97), MacFarland ('97), Erlanger ('97), Griffin ('99), Coe ('99), Linville (1900).

2. The cleavage centrosomes come from the egg centrosome; Van Beneden ('87), Wheeler ('95); all cases of normal and artificial parthenogenesis.

3. The cleavage centrosomes come from both egg and sperm centrosomes; Fol ('91), Guignard ('91), Blanc ('93), Conklin ('94), Carnoy and Lebrun ('99).

4. The cleavage centrosomes come from neither egg nor sperm centers but are new formations; Foot ('97), Lillie ('97), Child ('97), Mead ('98).[1]

It is probable that some of these conflicting views may be attributed to erroneous observations, and the writers who have maintained the first view have in general explained all others as due to this one cause, but on the other hand some of the evidence in favor of these other views cannot be thus lightly brushed aside. As long as this question remained on a purely observational basis no one seems to have seriously considered that there might be an element of truth in more than one of these views and that the cleavage centrosomes might arise differently in differ-

[1] For references see Wilson, "The Cell in Development and Inheritance," 1900.

ent animals or even in the same animal under different conditions.

The experiments of R. Hertwig, Morgan, Loeb and Wilson have shown that the unfertilized egg is capable of giving rise to cleavage centrosomes and spindles, while the observations of Delage, Boveri and others on merogeny have proven that a normal mitotic figure may appear in connection with the sperm nucleus in enucleated egg fragments. Under these circumstances it ought to be possible, by slightly altering normal conditions, to bring about the formation of a spindle in connection with each germ nucleus.

In the summer of 1901, while at the Marine Biological Laboratory at Woods Holl, I undertook some experiments on the eggs of *Crepidula* in order among other things to test this possibility. Since this animal is one which does not conform to the prevalent view as to the origin of the cleavage centrosomes it seemed all the more favorable for such work. As the eggs of these gasteropods are fertilized while still in the oviduct I have found it impracticable to experiment with unfertilized eggs but have worked entirely with eggs into which a spermatozoön had already entered.

The normal course of the fecundation in this animal may be briefly recalled: After both polar bodies have been extruded the egg nucleus lies at the animal pole in an area of cytoplasm while the sperm nucleus lies in the yolk near the periphery of the egg and usually near the vegetative pole. Then the egg centrosome which is left in the egg at the close of the second maturation division, rapidly disappears and in its place is left the large egg aster or sphere. At no stage is there a clearly marked sperm centrosome, but a radiating cytoplasmic figure, the sperm aster, develops in connection with the sperm nucleus and these two migrate through the yolk until they come into contact with the egg nucleus and sphere, immediately under the polar bodies. Here the egg and sperm spheres fuse and at their periphery two separate and independent centrosomes appear which ultimately come to lie at opposite poles of the first cleavage spindle. It is probable that one of these centrosomes comes from the egg sphere and the other from the sperm sphere though there is no

positive evidence that they are directly derived from egg and sperm centrosomes.

If now the eggs of *Crepidula plana* which have given off the second polar body but a short time before are brought for four hours into a 1 per cent. solution of NaCl in normal sea water

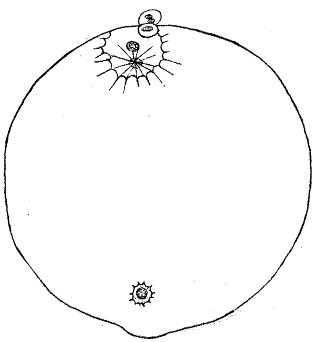

FIG. 1.

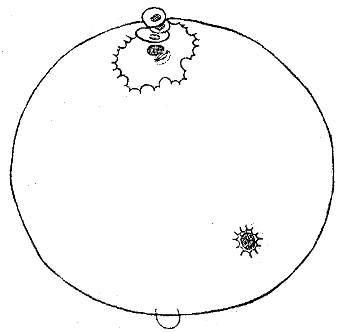

FIG. 2.

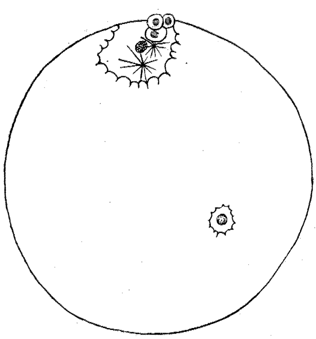

FIG. 3.

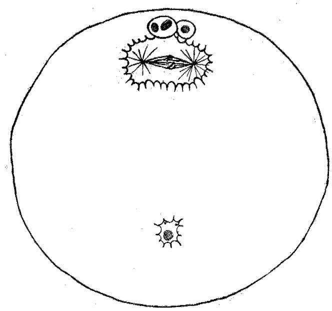

FIG. 4.

FIGS. 1–4. Eggs of *Crepidula plana* treated with 1 per cent. NaCl in sea water for four hours, viewed from the side. In all the eggs the polar bodies are at the upper pole, the egg nucleus and centrosome, or spindle, immediately below this, while the sperm nucleus lies in a small area of cytoplasm near the lower pole. Various stages in the formation of the egg spindle are shown.

they will present the appearances shown in Figs. 1–12. These figures are camera drawings of eggs in different stages of the formation of the first cleavage spindle and all are from the same experiment.

An examination of these figures shows that various stages in the division of the egg centrosome occur (Figs. 1–5) leading to

the formation of a perfect spindle of small size. There can be no doubt that the centrosomes of this spindle are derived from the egg centrosome. The sperm nucleus is at this time far removed from the egg nucleus and is closely surrounded by yolk; no astral radiations are found in connection with it, though one

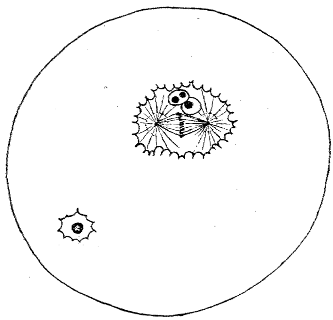

FIG. 5.

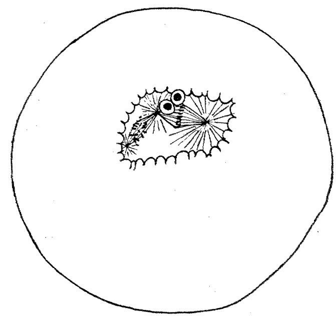

FIG. 6.

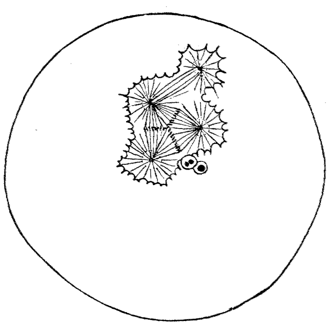

FIG. 7.

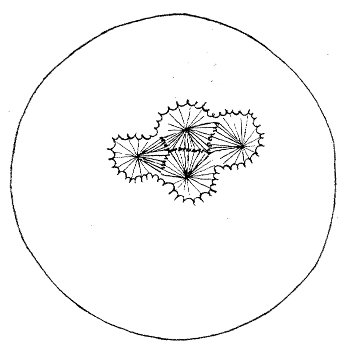

FIG. 8.

FIGS. 5–8. NaCl eggs of *C. plana* viewed from the animal pole; the two polar bodies are shown nearly over the spindles in the first three figures. In Fig. 5 the sperm nucleus is still some distance from the egg spindle; Fig. 6 shows an egg spindle and a sperm spindle joined at one pole; Figs. 7 and 8 show stages in the formation of a tetraster from the egg and sperm spindles.

or two granules which lie close to the nuclei may possibly represent centrosomes (Fig. 4).

Unfortunately a gap occurs at this stage in my material; in all the preceding figures (1–5) the sperm nucleus is small and densely chromatic and is far removed from the animal pole; in the succeeding figures (6–12) the sperm nucleus has been re-

solved into chromosomes which lie near those of the egg. In all cases there are two spindles present which are usually united into a tetraster though they may be more or less independent of each other. In some eggs yolk spherules separate the two spindles so that a tetraster is not formed (Figs. 9, 10), while in others an incomplete tetraster is formed (Figs. 6, 7); in still

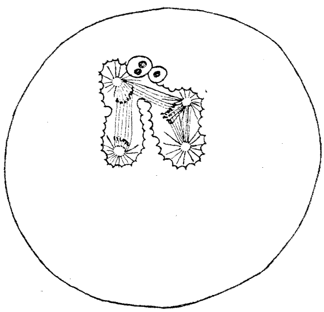

FIG. 9.

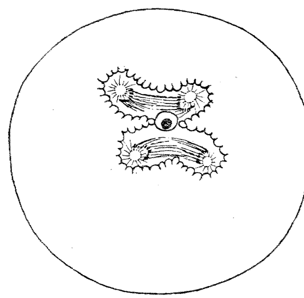

FIG. 10.

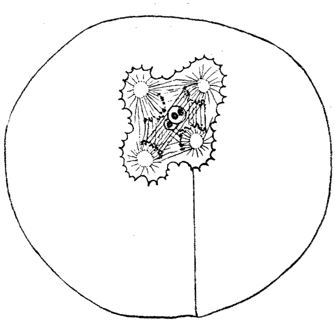

FIG. 11.

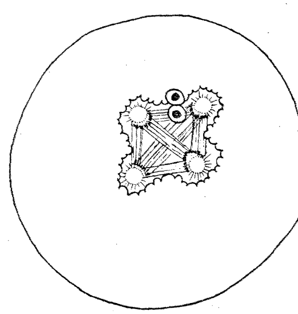

FIG. 12.

FIGS. 9–12. NaCl eggs of *C. plana*, viewed from the animal pole; the polar bodies lie above the spindles in all the figures. Fig. 9. Egg and sperm spindles united at one pole. Fig. 10. The two spindles quite separate. Figs. 11 and 12. Complete tetrasters in different phases of the separation of the chromosomes.

others the tetraster is complete, each of the four poles being united to the other three (Figs. 8, 11, 12).

Although I have not seen the genesis of the sperm spindle there seems to be no reason for doubting that it is formed essentially as the egg spindle is and that the two are at first wholly independent; only later do secondarily formed fibers connect one

or both of its poles with those of the egg spindle. Since under normal conditions one of the cleavage centrosomes of *Crepidula* arises in connection with the egg and the other with the sperm nucleus the only respect in which these experimental results differ from the normal is that in the former the centrosomes divide while the nuclei are still far apart thus giving rise to two spindles or to a tetraster, whereas in the latter these centrosomes do not appear until after the germ nuclei and spheres have met and they do not divide until the prophase of the second cleavage.

In such a case as this we have essentially the same phenomenon as is found in dispermic echinoderm eggs (Driesch, Boveri, Wilson) save that in the former the two additional centrosomes are not derived from an additional spermatozoön but come from the egg centrosome. Of this fact there cannot be a particle of doubt and it seems to me to shed light upon the much discussed question as to the source of the cleavage centrosomes of different animals under normal conditions.

It has evidently been a mistake to suppose that the cleavage centrosomes could arise in but a single way and that all animals must conform to this single type. Under experimental conditions the cleavage centrosomes may arise in one and the same animal in connection with the sperm nucleus, in connection with the egg nucleus or, as I have shown in connection with both of these nuclei, while it is possible that they may arise *de novo* anywhere in the egg cytoplasm, though this latter view is by no means so well supported by evidence as are the former ones (see Conklin 1902). It is highly probable therefore that the source of the cleavage centrosomes may differ in different animals, or even in the same animal under different conditions.

It is interesting to note that this whole discussion as to the supposed importance of the source of the cleavage centrosomes had its origin in the thought that the sex cells were in themselves incomplete and incapable of development save as each complemented the other (Boveri '87, '91), or in the rival notion that the centrosomes were the bearers of heritable qualities (Fol '91). In the light of recent experimental work both of the these views are seen to be untenable and the subject has therefore lost most of its interest and significance.